

FIG. 1A

| PEPTIDE | SEQUENCE | ANTIBODY DILUTIONS | | | | |
|-----------------|---|--------------------|----------|----------|----------|----------|
| | | 1.00E+03 | 5.00E+03 | 1.00E+04 | 5.00E+04 | 1.00E+05 |
| Th' | XX-XXX-Thr-XX-XXX-Cys | 1.92 | 1.32 | 0.54 | 0.34 | 0.07 |
| Ser-Thr | XX-XXX-Ser-Thr-XXX-XXX-Cys | 0.11 | 0.05 | 0.01 | 0.00 | 0.00 |
| Threonine mix | 18 phospho-Thr peptide | 1.84 | 1.13 | 0.40 | 0.26 | 0.10 |
| Serine mix | 38 phospho-Ser peptide | 0.12 | 0.04 | 0.02 | 0.02 | 0.01 |
| Akt-Thr308-P | Ile-Lys-Ala-Gly-Ala-Thr-Met-Lys-Thr-Phe-Cys-Gly-Thr-Pro | 1.18 | 0.65 | 0.24 | 0.13 | 0.03 |
| (SEQ ID NO: 1) | (SEQ ID NO: 3) | | | | | |
| APP1-Thr688-P | Asp-Ala-Ala-Ala-Thr-Pro-Lys-Lys-Ala-His-Leu-Ser-Lys-Cys | 0.14 | 0.03 | 0.01 | 0.01 | 0.01 |
| (SEQ ID NO: 2) | | | | | | |
| C3-P | Asp-Thr-Gln-Ile-Val-Asp-Ala-Thr-Phe-Val-Gly-Thr-Pro-Phe-Cys | 1.71 | 1.13 | 0.39 | 0.22 | 0.03 |
| (SEQ ID NO: 4) | (SEQ ID NO: 5) | | | | | |
| Cdk5-Thr167-P | His-Gln-VaL-VaL-Thr-Asp-Tyr-Asp-Cys | 1.77 | 1.15 | 0.41 | 0.27 | 0.08 |
| (SEQ ID NO: 6) | | | | | | |
| CAMIV-Thr188-P | His-Gln-VaL-VaL-Thr-Asp-Tyr-Asp-Cys | 1.79 | 1.36 | 0.63 | 0.40 | 0.09 |
| (SEQ ID NO: 7) | | | | | | |
| CDC2-Thr157-P | Ile-Pro-Gly-VaL-Ven-His-Glu-Gly-VaL-VaL-Thr-Leu-Cys | 1.02 | 0.56 | 0.14 | 0.08 | 0.03 |
| (SEQ ID NO: 8) | | | | | | |
| CDK2-Thr159-P | Gly-VaL-Ven-His-Glu-Gly-VaL-VaL-VaL-Thr-Leu-Cys | 1.88 | 1.79 | 0.61 | 0.44 | 0.08 |
| (SEQ ID NO: 9) | | | | | | |
| p70S6K-Thr389-P | Asn-Gln-Ala-Phe-Lys-Lys-Thr-Thr-Asp-Gly-Val-Ala-Phe-Lys-Lys-Cys | 1.69 | 1.44 | 0.62 | 0.39 | 0.08 |
| (SEQ ID NO: 10) | | | | | | |
| PKCalpha-P | Lys-Glu-His-Met-Met-Met-Ala-Asp-Gly-Val-Thr-Thr-Ala-Thr-Phe-Cys | 1.82 | 1.83 | 0.94 | 0.68 | 0.18 |
| (SEQ ID NO: 11) | | | | | | |
| ERK2-P | Asp-His-Thr-Gly-Phe-Leu-Thr-Glu-Tyr-Val-Ala-Thr-Ala-Tyr-Cys | 1.56 | 1.19 | 0.51 | 0.30 | 0.07 |
| (SEQ ID NO: 12) | | | | | | |
| Myc Ser581/S2-P | Glu-Leu-His-Pro-Thr-Pro-Pro-Lys-Gly-Pro-Ser-Ala-Ala-Ser-Cys | 0.11 | 0.05 | 0.03 | 0.02 | 0.02 |
| P38-2P | (SEQ ID NO: 13) | 0.64 | 0.30 | 0.08 | 0.08 | 0.04 |
| JN5-2P | Ser-Phe-Met-Met-Thr-Pro-Tyr-Val-Val-Thr-Ala-Tyr-Tyr-Cys | 1.40 | 0.44 | 0.12 | 0.07 | 0.03 |
| (SEQ ID NO: 13) | | | | | | |

FIG. 1B

| PEPTIDE SEQUENCE | phospho-Thr Reactivity |
|--|------------------------|
| XXXXXXSXXXXXX | — |
| XXXXYXXXXX | — |
| XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14) | ++ |
| XXXRSXS*XPXXXX (SEQ ID NO:15) | — |
| XXXRSXSXPXXXX (SEQ ID NO:16) | — |
| XXXXXPXS*/T*PXXXXX (SEQ ID NO:17) | ++ |
| XXXXXPXS/TPXXXXX (SEQ ID NO:18) | — |
| XXXXXTXXXXXX | +++ |
| XXXXXXS/TXXXXXX | — |
| 21 phospho-Thr peptides mixture | +++ |
| 38 phospho-Ser peptides mixture | — |
| 30 phospho-Tyr peptides mixture | — |
| NEB LIBRARY | |
| X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19) | +++ |
| X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20) | ++ |
| X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21) | +++ |
| X-X-X-X-R/K-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22) | +++ |
| X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:23) | +++ |
| X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24) | +++ |
| X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-C (SEQ ID NO:25) | +++ |
| X-X-X-X-X-S*/T-P-X-X-X-X-X-X-C (SEQ ID NO:26) | ++ |
| X-X-X-X-X-T*-X-X-X-X-X-X-C | +++ |
| X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27) | ++ |
| X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28) | — |
| X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-C (SEQ ID NO:29) | ++ |
| ANTIBODY REACTIVITY | |
| +++ | very strong |
| ++ | strong |
| + | weak |
| - | very little |
| ELISA O.D. | |
| > 2 | |
| 1 - 2 | |
| 0.2 - 1 | |
| < 0.2 | |

FIG. 1C

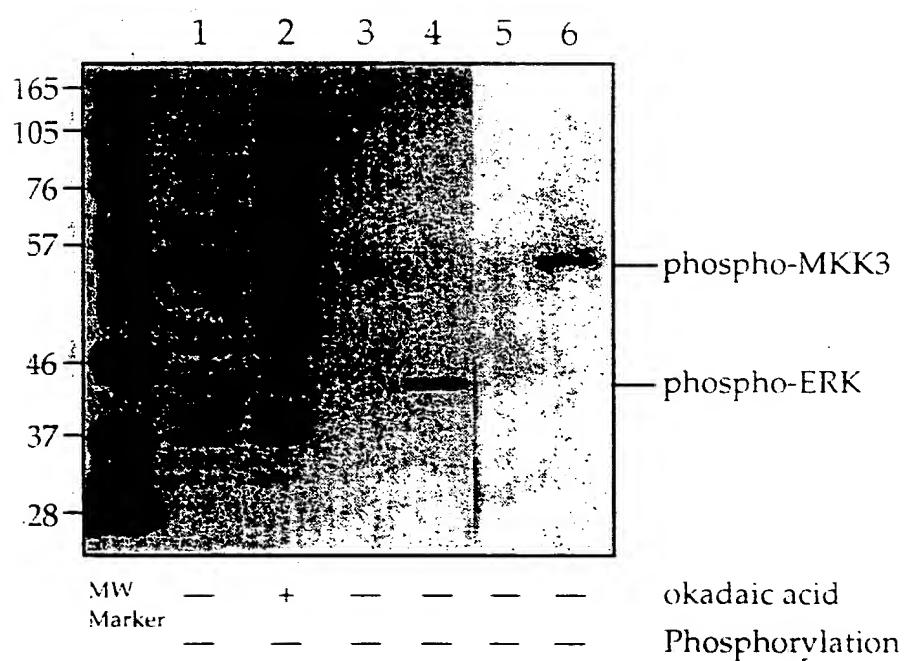


FIG. 1D

| | | -5-4-3-2-1 X X X X X Ser*/Thr* | | | | +1+2+3+4+5 X X X X X | | |
|------------|---|-----------------------------------|----|----|----|-------------------------|----|----|
| Amino Acid | Fixed AA position relative to phospho-Ser*/Thr* | S*/T* | | | | +1 | +2 | +3 |
| | | -4 | -3 | -2 | -1 | | | |
| Ala | + | + | + | + | + | + | + | + |
| Cys | + | + | + | + | + | + | + | + |
| Asp | + | + | + | + | + | + | + | + |
| Glu | + | + | + | + | + | + | + | + |
| Phe | + | + | + | + | + | + | + | + |
| Gly | + | + | + | + | + | + | + | + |
| His | + | + | + | + | + | + | + | + |
| Ile | + | + | + | + | + | + | + | + |
| Lys | + | + | + | + | + | + | + | + |
| Leu | + | + | + | + | + | + | + | + |
| Met | + | + | + | + | + | + | + | + |
| Asn | + | + | + | + | + | + | + | + |
| Pro | + | + | + | + | + | — | + | + |
| Gln | + | + | + | + | + | + | + | + |
| Arg | + | + | + | + | + | + | + | + |
| Ser | + | + | + | + | + | + | + | + |
| Thr | + | + | + | + | + | + | + | + |
| Val | + | + | + | + | + | + | + | + |
| Trp | + | + | + | + | + | + | + | + |
| Tyr | + | + | + | + | + | + | + | + |

FIG. 2A

| PEPTIDE | SEQUENCE | | | ANTIBODY DILUTIONS | | |
|------------------|--|--|--|--------------------|-----------------|-----------------|
| | | | | 1.00E+03 | 5.00E+03 | 1.00E+04 |
| | | | | 1.00E+04 | 5.00E+04 | 1.00E+05 |
| | | | | 5.00E+05 | 1.00E+06 | |
| PSGP-P | X-X-X-X-Pro-X-Ser*-Thr*-Pro-X-X-X-X-Cys | | | | | |
| (SEQ ID NO. 27) | | | | 1.82 | 1.97 | 1.74 |
| | | | | | | 1.40 |
| | | | | | | 0.70 |
| | | | | | | 0.35 |
| | | | | | | 0.08 |
| Threonine mix | 18 Phospho-Thr peptide mix | | | | | |
| | | | | 1.97 | 1.37 | 0.67 |
| | | | | | | 0.36 |
| | | | | | | 0.13 |
| | | | | | | 0.07 |
| | | | | | | 0.05 |
| Ser/Thr | X-X-X-X-Ser/Thr-X-X-X-X-Cys | | | | | |
| (SEQ ID NO. 28) | | | | 0.14 | 0.03 | 0.01 |
| | | | | | | 0.00 |
| | | | | | | 0.00 |
| | | | | | | 0.00 |
| RB Thr373-P | Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Ala-Gly-Thr-Val-Met-Asn-Thr-Cys | | | | | |
| (SEQ ID NO. 30) | | | | 2.07 | 2.17 | 1.70 |
| | | | | | | 1.20 |
| | | | | | | 0.48 |
| | | | | | | 0.18 |
| | | | | | | 0.03 |
| MKK3-Thr-P | Ser-Val-Ala-Lys-Thr*-Met-Asp-Ala-Gly-Cys | | | | | |
| (SEQ ID NO. 31) | | | | 0.06 | 0.04 | 0.01 |
| | | | | | | 0.00 |
| | | | | | | 0.00 |
| | | | | | | 0.00 |
| PKCalpha-P | Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Ala-Pro-Lys-Lys-Cys | | | | | |
| (SEQ ID NO. 3) | | | | 0.05 | 0.02 | 0.01 |
| | | | | | | 0.00 |
| | | | | | | 0.01 |
| | | | | | | 0.00 |
| P70 S6K-Thr389-P | Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr*-Tyr-Val-Ala-Pro-Lys-Lys-Cys | | | | | |
| (SEQ ID NO. 8) | | | | 0.11 | 0.05 | 0.01 |
| | | | | | | 0.00 |
| | | | | | | 0.01 |
| | | | | | | 0.00 |
| cdk4-Thr172-P | Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr*-Pro-Val-Val-Val-Lys-Cys | | | | | |
| (SEQ ID NO. 32) | | | | 2.07 | 2.21 | 2.01 |
| | | | | | | 1.55 |
| | | | | | | 0.69 |
| | | | | | | 0.31 |
| | | | | | | 0.07 |

FIG. 2B

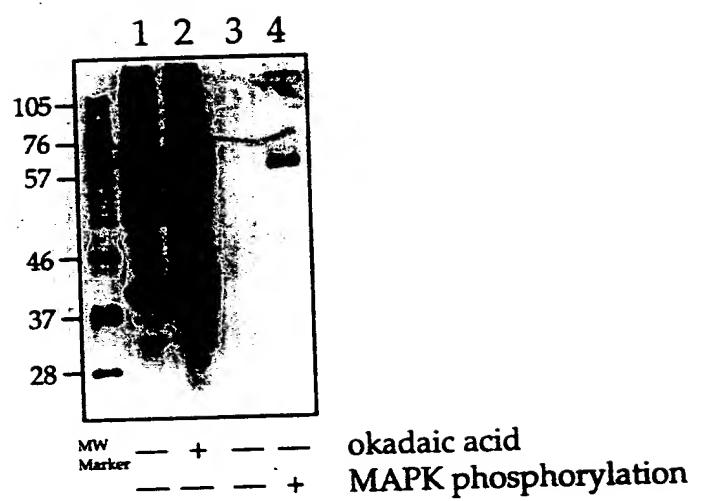


FIG. 3A

| PEPTIDE | SEQUENCE | | | | ANTIBODY DILUTIONS | |
|----------------|--|----------|----------|----------|--------------------|----------|
| | | 1.00E+03 | 8.00E+03 | 1.00E+04 | 5.00E+04 | 1.00E+05 |
| 14-3-3 BM-P | X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-Cys (SEQ ID NO: 33) | 2.41 | 2.16 | 1.49 | 1.15 | 0.44 |
| 14-3-3 BM | X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-Cys (SEQ ID NO: 34) | 0.07 | 0.03 | 0.02 | 0.03 | 0.03 |
| CDC25-Ser216-P | Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Ala-Leu-Ala-Arg-Cys (SEQ ID NO: 35) | 2.35 | 2.08 | 1.49 | 1.05 | 0.33 |
| CDC25-Ser216 | Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Ala-Leu-Ala-Arg-Cys (SEQ ID NO: 36) | 0.05 | 0.02 | 0.03 | 0.03 | 0.04 |
| Bad-Ser112-P | Thr-Ala-Ser-Ala-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Gly-Gly-Cys (SEQ ID NO: 37) | 1.59 | 0.43 | 0.10 | 0.03 | 0.01 |
| Bad-Ser112 | Thr-Ala-Ser-Ala-His-Ser-Ser-Tyr-Pro-Ala-Gly-Gly-Gly-Cys (SEQ ID NO: 38) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Bad-Ser136 | Phe-Ala-Gly-Ala-Ser-Ala-Ser-Ala-Pro-Pro-Ala-Leu-Tyr-Ala-Cys (SEQ ID NO: 39) | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| Bad-Ser136-P | Phe-Ala-Gly-Ala-Ser-Ala-Pro-Pro-Ala-Aaa-Leu-Tyr-Ala-Cys (SEQ ID NO: 40) | 3.25 | 1.86 | 0.73 | 0.51 | 0.07 |
| | | | | | | 0.03 |

FIG. 3B

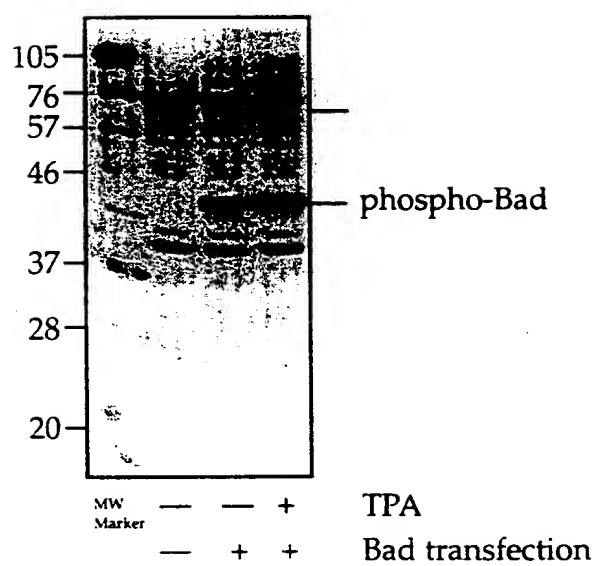


FIG. 4A

| PEPTIDE | SEQUENCE | MONOCLONAL ANTIBODIES |
|----------------------|--|-----------------------|
| Ser/Thr-Pro-P | X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-Cys (SEQ ID NO: 26) | 6B6 1.774 0.731 |
| ProXSer/ThrPro-P | X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-Cys (SEQ ID NO: 27) | 6A9 0.924 0.788 |
| ProXSer/ThrPro-P | X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-Cys (SEQ ID NO: 41) | 0.02 0.063 |
| ProXSer/ThrProXArg-P | X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-Arg-Lys-X-X-X-Cys (SEQ ID NO: 42) | 1.955 1.275 |
| Thr-P | X-X-X-X-X-X-Thr-X-X-X-X-Cys | 0 .. |
| Ser-P | X-X-X-X-X-X-Ser-X-X-X-X-Cys | 0.031 0.088 |
| Ser/Thr | X-X-X-X-X-X-Ser/Thr-X-X-X-X-Cys | 0.021 0.088 |
| Tyr-P | X-X-X-X-X-X-Tyr-X-X-X-X-X-Cys | 0.023 0.072 |
| Rb (Ser795)-P | Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser-Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO: 43) | 0.032 0.124 |
| Rb (Thr373)-P | Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30) | 3.338 3.503 |
| Rb (Thr373) | Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 44) | 0.02 0.073 |

FIG. 4B

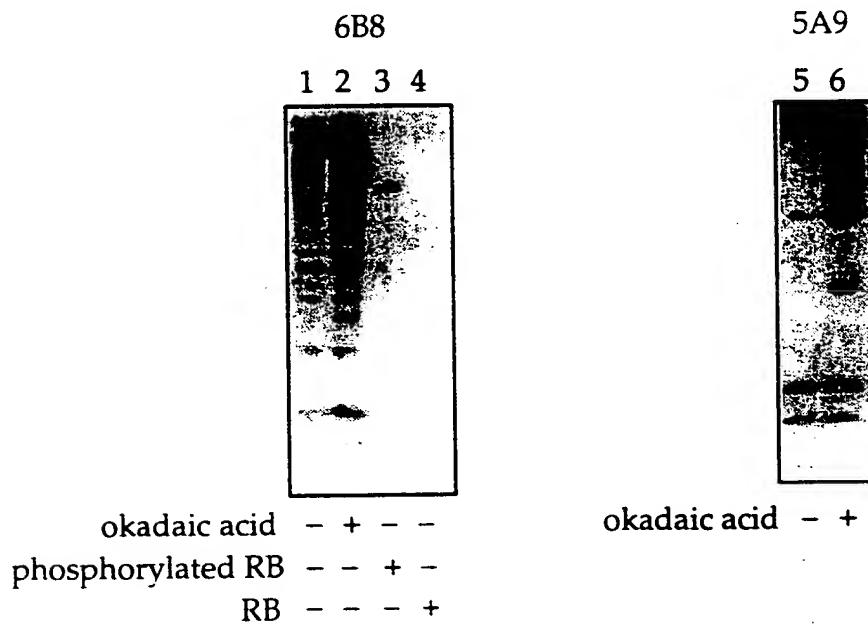


FIG. 5A

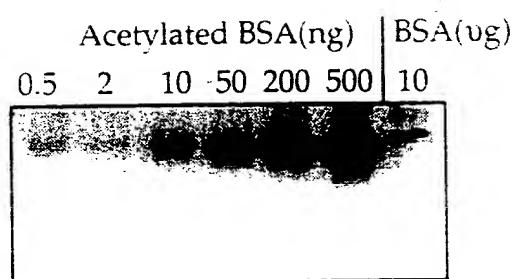


FIG. 5B



1 2 3 4

FIG. 5C



5 6 7 8

FIG. 5D



9 10 11 12

Phospho-Akt Substrate Antibody

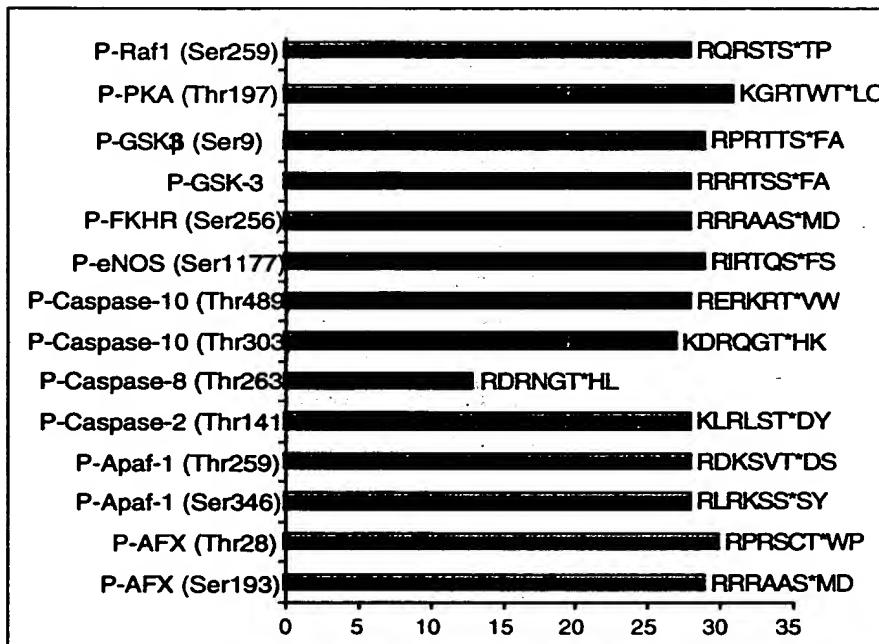


Figure 6: Signal to noise ratio of ELISA readings using Phospho-Akt Substrate Antibody with phosphopeptides of Akt substrates vs. non-phospho-peptides of Akt substrates.



Figure 7: Western analysis of calyculin A-treated A431 cells using Phospho-Akt Substrate Antibody.

Phospho-PKA Substrate Antibody

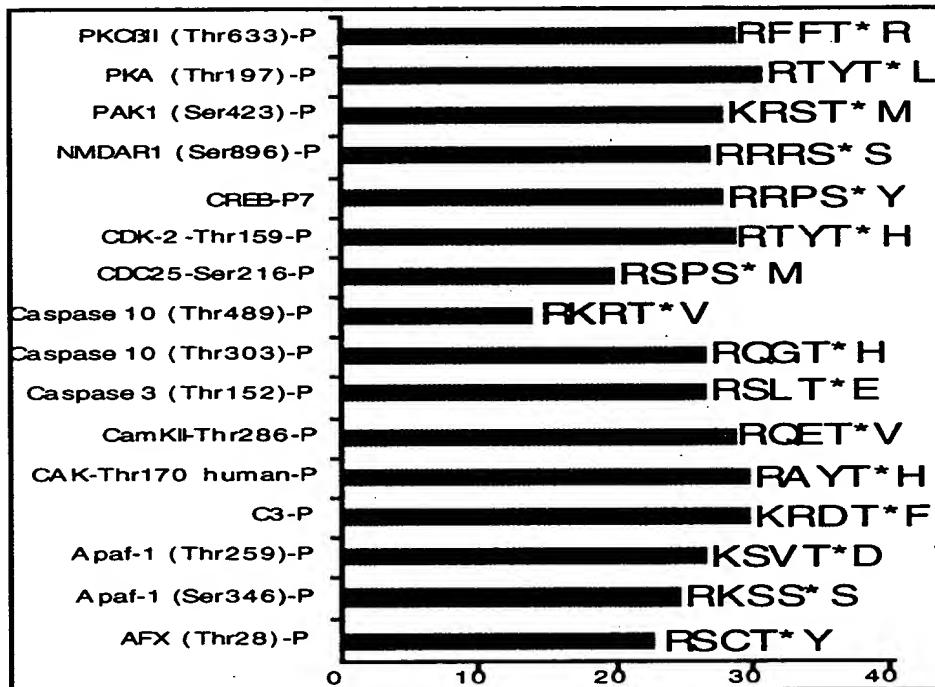


Figure 8: Signal to noise ratio of ELISA reading using phospho-PKA substrates antibody against peptides have Arginine or Lysine at -3 position.

Phospho-PKA Substrate Antibody



Figure 9: Western analysis of calyculin A-treated A431 cells using Phospho-PKA Substrate Antibody.

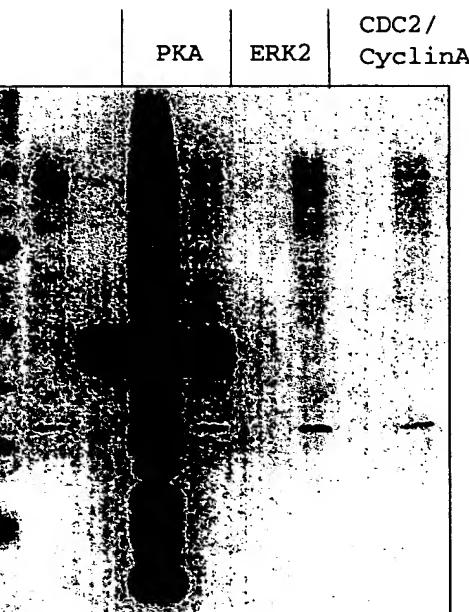


Figure 10: Western analysis of A431 cell extracts phosphorylated by protein kinase A, ERK2 or CDC2/cyclinA in vitro using Phospho-PKA Substrate Antibody.

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Phospho-Serine/Threonine Phenylalanine Antibody

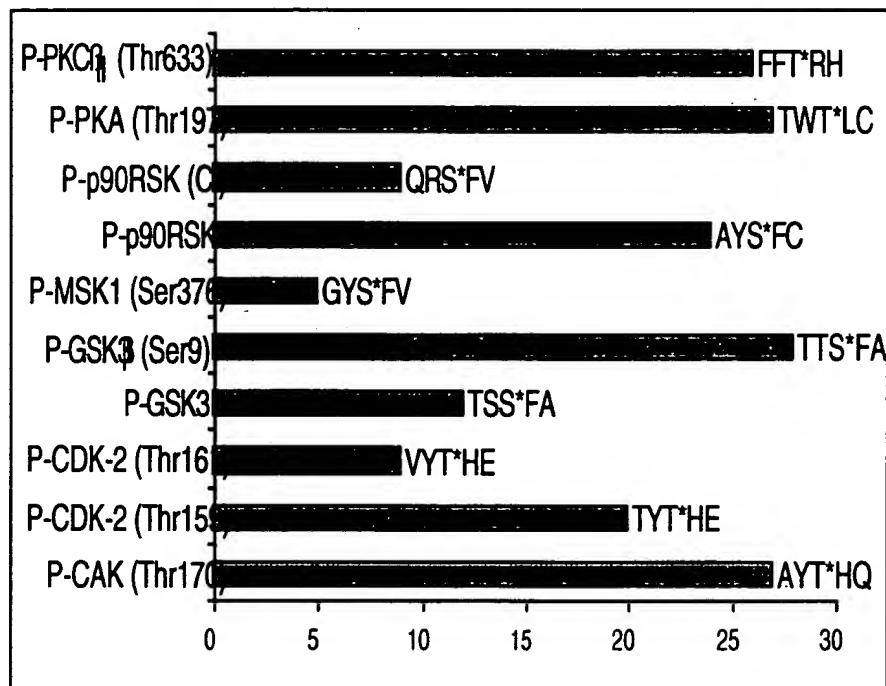


Figure 11: Signal to noise ratio of ELISA reading using phospho-Serine/threonine phenylalanine antibody against the peptides surrounded by phenylalanine, tyrosine or tryptophan.



Figure 12: Western analysis of calyculin A-treated A431 cells using phospho-Serine/phenylalanine substrates antibody.

- +
calyculin A